

Temperature and Moisture Status Affect Afterripening of Leafy Spurge (*Euphorbia esula*) Seeds

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Increasing the germination potential of dormant seeds in a population over time generally requires afterripening. Research was conducted to study the relationship between temperature and seed moisture content on afterripening of dormant leafy spurge seeds. Germination of nonafterripened seeds was 59 and 36% after 21 d for the Harwood and Fargo populations, respectively. Germination of 85 to 87% and 58 to 62% was obtained for the Harwood and Fargo populations, respectively when afterripened for 12 to 24 wk under the most effective conditions of 30 C and 2.6% seed moisture; increasing the afterripening temperature to 45 C did not increase germination. Germination decreased slightly at 30 C as the seed moisture content increased to 5.6%, but germination still exceeded that of nonafterripened seeds. Afterripening at 30 C with a seed moisture content of $\geq 9\%$ greatly decreased germinability due to seed ageing. A temperature of 5 C was effective for afterripening when the moisture content was 3.3%, but germination was still slightly less than for the low moisture content seeds afterripened at 30 C. Afterripening seeds with 6 to 13% moisture at 5 C generally did not increase germination compared with the control, but did not result in seed ageing.

Nomenclature: Leafy spurge, *Euphorbia esula* L. EPHES.

Key words: Ageing, dormancy, germination, weed.

Leafy spurge is a noxious perennial weed in 35 states and six Canadian provinces (CAB 2004). Its annual economic impact in the four-state region of North and South Dakota, Montana, and Wyoming is about \$130 million (Leitch et al. 1996). Leafy spurge reproduces vegetatively from adventitious crown and root buds and sexually from seeds. Applied and fundamental aspects of vegetative reproduction have been investigated for many years because this trait makes it particularly difficult to control. Recently, an emphasis has been placed on transcriptome analysis of dormancy in buds (Horvath et al. 2006; Jia et al. 2006).

Seed production, dispersal, longevity, and dormancy are all factors in the persistence and spread of leafy spurge. Mature plants produce about 250 seeds per shoot that can be forcefully dispersed for distances up to 4.5 m (Bakke 1936; Selleck et al. 1962). In addition, seeds are dispersed by moving water, wildlife, and livestock (Blockstein et al. 1987; Lacey et al. 1992; Selleck et al. 1962). Leafy spurge seeds have great longevity in the soil. Brown and Porter (1942) determined that leafy spurge seeds buried in the soil retained nearly full viability over a period of 3 yr. Other research demonstrated the need for long-term management programs because treatments that eliminated or reduced leafy spurge seed production for 3 to 8 yr still resulted in viable seeds in the soil for reestablishment (Bowes and Thomas 1978; Kirby et al. 2003).

Delayed germination resulting from seed dormancy provides a means to buffer invasive annual and perennial plant species against local extinction resulting from variable environments and years of reproductive failure resulting from chemical and biological control measures. Dormancy in leafy spurge populations varies from little or no dormancy (Bakke 1936) to long periods of dormancy (Bowes and Thomas 1978). Dormancy in leafy spurge is classified as nondeep physiological dormancy, which implies the embryo is fully developed at maturity (Baskin and Baskin 1998). Seeds with nondeep physiological dormancy can be afterripened over

some period of time through exposure of seeds to a set of environmental conditions. Seeds with nondeep physiological dormancy normally respond to warm or cool stratification under dry or moist conditions to transition from a dormant to a less or nondormant state capable of a relatively rapid onset and rate of germination (Baskin and Baskin 1998). I examined the general effect of afterripening on germination of leafy spurge seeds and discovered that afterripening for 12 to 24 wk under several conditions generally provided for increased germinability compared to nonafterripened seeds (Foley 2004). In the aforementioned study, the conditions of temperature and seed moisture were rudimentarily controlled. Thus, the objective of this research was to evaluate if a relationship exists between temperature and seed moisture content for afterripening of dormant leafy spurge seeds.

Material and Methods

Leafy spurge has indeterminate flowering and mature seeds dehisce naturally and forcefully from the fruit coats in the afternoon on warm, dry days. We harvested plants by hand in early morning to early afternoon at the peak of seed production in late June to early July 2006 from field populations in Harwood, ND (46°58'45"N, 96°52'50"W) and Fargo, ND (46°52'37" N, 96°47'23"W) to obtain a maximum amount of mature seeds. Plants were air dried in open paper bags for 7 to 20 d. All seeds were cleaned by hand, separated into six fractions by weight using a seed blower,¹ and then stored at -20 C to maintain dormancy. Seed fractionation was done because the populations harvested in the manner described above contained a proportion of nonviable and less mature seeds as judged by seed color (Wicks and Derscheid 1964) and our baseline germination tests (unpublished data). To reduce the potential for confounding due to nonviable and immature seeds, fraction 4 seeds were used for these controlled environment experiments. Viability of seeds in fractions 3 to 6 was 95%, but fraction 4 was selected for experiments because it contained the largest proportion of seeds (28%). The average weight of fraction 4 seeds for Harwood and Fargo populations was 3.0 mg seed⁻¹. Fraction 4 seeds were obtained with a 3.8-

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Table 1. Average seed moisture content provided by saturated salt solutions during afterripening at two temperatures.

Saturated salt	5 C	30 C
	% moisture	
ZnCl ₂	3.3 ± 0.28	2.6 ± 0.57
Potassium acetate	6.1 ± 0.16	4.2 ± 0.44
MgCl ₂	6.7 ± 0.25	5.6 ± 0.26
NaCl	10.2 ± 0.19	9.0 ± 0.41
KCl	13.0 ± 0.59	10.7 ± 0.52

cm seed blower separation tube using an airflow setting of 4.0 to 4.5.

Seeds for germination and fresh weight (FW) and dry weight (DW) determinations were placed in perforated 0.5 ml microcentrifuge tubes and equilibrated to various moisture contents in sealed 450-ml jars over saturated salt solutions at 5 and 30 C for 6, 12, and 24 wk. The saturated salt solutions were ZnCl₂, potassium acetate (KAc), MgCl₂, NaCl, and KCl, and the relative humidity of these constant-humidity chambers was taken from Rockland (1960) and Winston and Bates (1960). The mean percent seed moisture content, which is a quantitative measure of the actual seed moisture content provided by each saturated salt solution, was determined three times during the course of afterripening on samples of 15 seeds. Seed FW was determined after the exterior surface was rapidly blotted dry with a soft tissue and the DW was determined after drying for several days at 80 C. Seed moisture was expressed on a percent DW basis (Table 1). Nonafterripened control seeds germinated at 6, 12, and 24 wk were maintained at -20 C prior to germination.

Seeds for each treatment were surface disinfected for 10 min with a 50% solution of commercial bleach (NaOCl) containing a drop of Triton X-100 surfactant and rinsed four times for 1 to 2 min with sterile distilled water. The surface disinfected seeds were dried for about 2 h to their original FW to de-gas chlorine (unpublished data).

Seeds were germinated in petri dishes containing sterile distilled water and lined with one Whatman #1 filter paper. Petri dishes were maintained at an alternating temperature of 30/20 C (8/16 h) in the dark in boxes lined with wet paper towel to maintain high relative humidity. Germination as judged visually by cracking of the seed coat was normally determined daily throughout the 1 to 21 days of incubation.

The initial experiment consisted of two populations, seed moisture levels provided by five saturated salt solutions, two temperatures for afterripening, three periods of afterripening, and 21 d of germination. For each combination of population, temperature, and afterripening, three replicate jars of seeds for each of the seed moisture levels provided by the five saturated salt solutions were incubated in the same controlled environment chamber. Each jar included 12 perforated microcentrifuge tubes containing 50 seeds. The experiment was a split plot with incubators serving as whole plots and jars as subplots. The experiment was repeated once. Observed germination percentages were fit to a three parameter logistic growth model $Y = \Phi_1 / \{1 + \exp[-(\Phi_2 - \Phi_3) / \Phi_3]\}$ where Φ_1 is the asymptotic germination rate, Φ_2 is the time at which a population reaches half its asymptotic germination, Φ_3 is the time elapsed between the time a population reaches one-half the asymptotic germination rate and the time it reaches three-fourths germination rate, and d is time in days. Parameter

estimates were determined using PROC NLIN in SAS and then compared using PROC GLM.

A follow-up experiment was conducted using both populations similar to the way described above, except the experiment was not repeated in time, there were 25 seeds per treatment, and only ZnCl₂ was used to examine the effects of afterripening for 12 wk at temperatures of 5, 30, 45, and 5/30 C (12/12 h).

Results and Discussion

The germination rate of the control, that is nonaafterripened leafy spurge seeds, was different for the Harwood and Fargo populations (Figure 1A). Germination for the nonaafterripened Harwood and Fargo seeds was $59 \pm 3.4\%$ and $36 \pm 2.9\%$ (Figure 1A), respectively, after 21 d. In a previous investigation, nonaafterripened seeds harvested in 1999 from the same Fargo, ND, population displayed 24% germination after 21 d (Foley 2004); slightly less than in 2006. The $12 \pm 3\%$ difference in germination between years is unremarkable and may be explained by use of fractionated seeds for the experiments in 2006 or differences in environmental conditions (Bannon et al. 1978; Selleck et al. 1962). Nonfractionated populations that contain a proportion of low weight seeds have lower overall germination (unpublished data; Hanson and Rudd 1933).

The germination of nontreated seeds from our populations (Figure 1A) falls within the range of germination for several Saskatchewan populations. Seeds for these populations were extracted from the soil and germinated under controlled conditions similar to those used in this experiment (Bowes and Thomas 1978), or were harvested from plants and germinated in soil (Selleck et al. 1962). In any case, germination ranged from 29 to 66% for nontreated seeds extracted from soil and 0.8 to 44% for seeds harvested from plants and germinated in soil. Germination, under controlled conditions, of leafy spurge seeds from another population in the vicinity of Fargo, ND, ranged from 54 to 63% (Hanson and Rudd 1933). The results from these investigations refute anecdotal evidence that suggests little seed dormancy in populations of leafy spurge (Bakke 1936). Seed dormancy also occurs in populations of wild poinsettia (*Euphorbia heterophylla* L.) and prostrate spurge [*Chamaesyce humistrata* (Engelm. ex Gray) Small] (Bannon et al. 1978; Krueger and Shaner 1982).

The initial analysis indicated no difference between replicates of the primary experiment so data were combined. Subsequent analysis revealed a significant effect for population (Figure 1B), temperature during afterripening (Figure 1C), moisture content of the seeds during afterripening (Figure 1D), and day of germination (data not shown) (Table 2). The main effect for the duration of afterripening was not significant, but this factor interacted with moisture content, temperature, and day (Table 2 and Figures 2 and 3). The significant effect of day, and interaction with other factors, is expected and understandable given the continuous increase of germination over the 21-d period for control and treated seeds (Figure 1).

When averaged over all factors and compared with the controls (Figure 1A and D), the low seed moisture provided by ZnCl₂ (2.6 to 3.3%) enhanced germination; the midlevel seed moisture provided by KAc and MgCl₂ (4.2 to 6.7%)

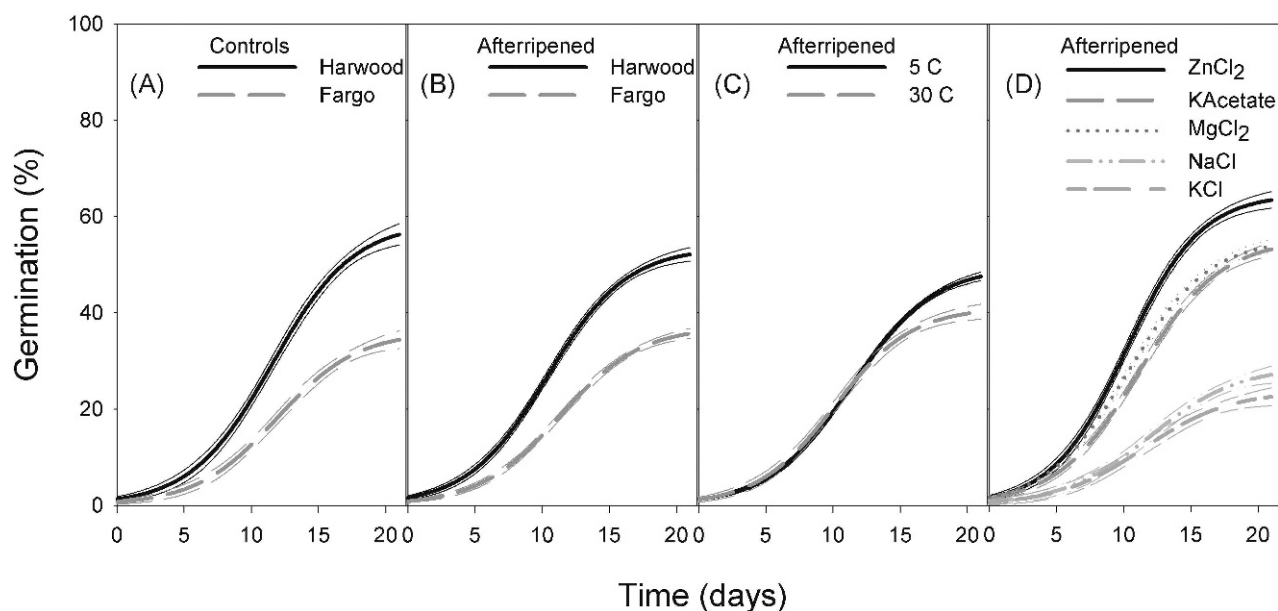


Figure 1. Predicted germination of leafy spurge seeds. (A) nonafterripened control seeds by population, (B) afterripened seeds by population; averaged over temperature and seed moisture, (C) afterripened seeds by temperature; averaged over population and seed moisture, and (D) afterripened seeds by seed moisture content (see Table 1), i.e., saturated salt solution; averaged over population and temperature. The 95% confidence intervals are depicted by the narrow lines. Equations are (A) $Y_H = 58.8/[1 + \exp[-(d - 11.6)/3.0]]$, $R^2 = 0.90$ and $Y_F = 36.1/[1 + \exp[-(d - 11.9)/3.0]]$, $R^2 = 0.77$; (B) $Y_H = 53.6/[1 + \exp[-(d - 10.4)/3.0]]$, $R^2 = 0.52$ and $Y_F = 37.2/[1 + \exp[-(d - 11.3)/3.0]]$, $R^2 = 0.51$; (C), $Y_{5C} = 49.6/[1 + \exp[-(d - 11.4)/3.1]]$, $R^2 = 0.72$ and $Y_{30C} = 41.1/[1 + \exp[-(d - 10.1)/2.9]]$, $R^2 = 0.33$; and (D) $Y_{ZnCl_2} = 64.8/[1 + \exp[-(d - 10.2)/2.8]]$, $R^2 = 0.73$; $Y_{KAc} = 55.3/[1 + \exp[-(d - 11.1)/3.1]]$, $R^2 = 0.75$; $Y_{MgCl_2} = 55.3/[1 + \exp[-(d - 10.3)/3.0]]$, $R^2 = 0.76$; $Y_{NaCl} = 28.8/[1 + \exp[-(d - 12.0)/3.2]]$, $R^2 = 0.36$; and $Y_{KCl} = 23.7/[1 + \exp[-(d - 11.5)/3.2]]$, $R^2 = 0.25$.

resulted in similar rates of germination to one another and to control seeds for the Harwood population; and the relatively higher seed moistures provide by NaCl and KCl (9 to 13%) resulted in similar rates of germination to one another that were relatively low (Figure 1D). However, temperature and the duration of afterripening modulate the seed moisture effect. For example, it first appears that 5 C is more conducive to afterripening than 30 C (Figure 1C). In fact, in almost all instances where the moisture content is 2.5 to 6.7% (Table 1), the rate of germination is greater when seeds from both populations are afterripened at 30 C (Figures 2A–C, 2F–H, and 2K–M and 3A–C, 3F–H, and 3K–M). However,

Table 2. Analysis of variance for the effect of population, duration of afterripening, seed moisture content, temperature, and day of germination on afterripening of leafy spurge seeds.

Source of variation	Pr > F
Population (Pop)	< 0.0001
Afterripening (AR)	0.2467
Pop * AR	0.2486
Moisture (Moist)	< 0.0001
Pop * Moist	< 0.0001
AR * Moist	< 0.0001
Pop * AR * Moist	0.0715
Temperature (Temp)	0.0413
Pop * Temp	0.5701
AR * Temp	0.5729
Pop * AR * Temp	0.02078
Moist * Temp	< 0.0001
Pop * Moist * Temp	< 0.0001
AR * Moist * Temp	0.00306
Pop * AR * Moist * Temp	0.0868
Day	< 0.0001
Pop * Day	< 0.0001
Pop * Salt * Day	< 0.0001
Moist * Temp * Day	< 0.0001
Pop * AR * Temp * Day	< 0.0001

as seed moisture increases to $\geq 9\%$ (Table 1), afterripening at 30 C over the course of 24 wk decreases the potential for germination, sometimes completely (Figures 2D–E, 2I–J, and 2N–O and 3D–E, 3I–J, and 3N–O). Seeds that did not germinate following afterripening under high-moisture, high-temperature conditions were not viable as determined by germination in 10 mM gibberellic acid (data not shown). Temperature and seed moisture levels also affect viability of wild poinsettia seeds in a similar manner (Bannon et al. 1978). For example, their viability was not affected when they were stored at 25 C for 36 wk with moisture levels below 7.7%, but their viability rapidly decreased when stored for 12 wk at 25 C with a moisture content of 10.8%.

In contrast to afterripening at 30 C, germination is not adversely effected when seeds with a relative high moisture level (10 to 13%) are afterripened for 24 wk at 5 C (Figures 2N–O and 3N–O). However, afterripening at 5 C and seed moisture contents > 3.3% generally provided little improvement in germination compared with the control (Figures 2B–E, 2G–J, and 2L–O and 3B–E, 3G–J, and 3L–O). Seeds of wild poinsettia stored at 5 C under dry conditions for 12 wk also retain their original level of dormancy as measured by germination (Bannon et al. 1978).

The most effective conditions for afterripening in this experiment were ≥ 12 wk at 30 C with the relatively low seed moisture content (2.6%) provided by ZnCl₂ (Figure 2A, 2F, and 2K and 3A, 3F, and 3K). The two populations still did not attain their full germination potential of 95% under these conditions. Thus, a subsequent experiment evaluated afterripening under low seed moisture conditions at a higher temperature (45 C) and an alternating temperature (5/30 C). The alternating temperature was selected because germination of leafy spurge and other *Euphorbia* spp. seeds generally is optimal with an alternating temperature (Bannon et al. 1978;

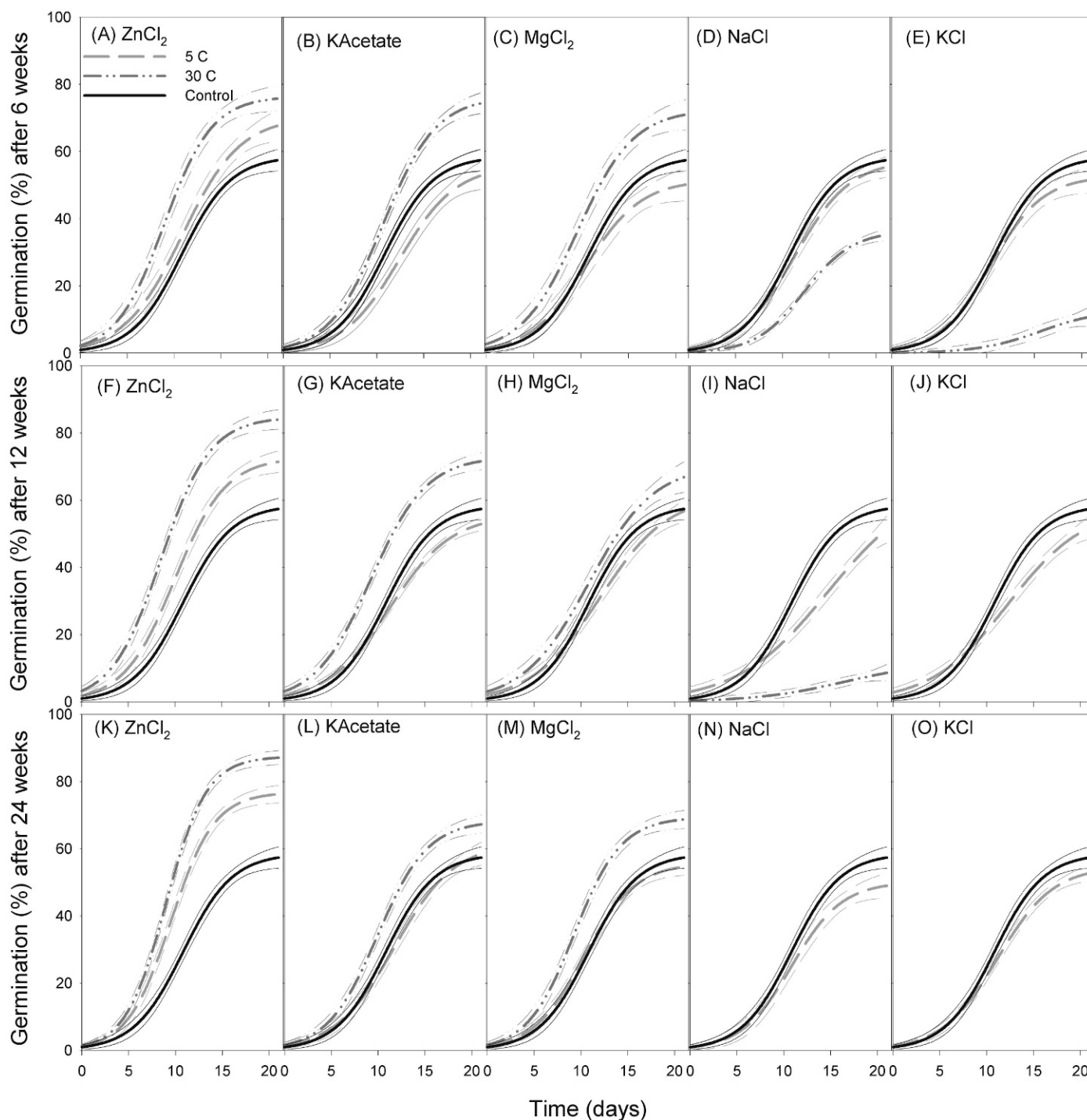


Figure 2. Predicted germination of leafy spurge seeds from the Harwood population afterterripened for (A–E) 6 wk, (F–J) 12 wk, and (K–O) 24 wk at 5 and 30 C with different seed moisture levels provided by saturated salt solutions (see Table 1). The nonafterripened control is included for comparison. The 95% confidence intervals are depicted by the narrow lines. Equations are (A) $Y_{5C} = 70.3/[1 + \exp[-(d - 10.9)/3.1]]$, $R^2 = 0.85$ and $Y_{30C} = 76.4/[1 + \exp[-(d - 8.9)/2.6]]$, $R^2 = 0.89$; (B) $Y_{5C} = 55.8/[1 + \exp[-(d - 12.4)/3.0]]$, $R^2 = 0.84$ and $Y_{30C} = 76.2/[1 + \exp[-(d - 10.6)/2.8]]$, $R^2 = 0.94$; (C) $Y_{5C} = 51.5/[1 + \exp[-(d - 10.5)/2.5]]$, $R^2 = 0.72$ and $Y_{30C} = 72.5/[1 + \exp[-(d - 9.6)/3.0]]$, $R^2 = 0.85$; (D) $Y_{5C} = 57.5/[1 + \exp[-(d - 11.3)/3.0]]$, $R^2 = 0.90$ and $Y_{30C} = 36.4/[1 + \exp[-(d - 12.3)/2.6]]$, $R^2 = 0.94$; (E) $Y_{5C} = 52.7/[1 + \exp[-(d - 10.4)/2.7]]$, $R^2 = 0.81$ and $Y_{30C} = 13.0/[1 + \exp[-(d - 15.6)/3.3]]$, $R^2 = 0.33$; (F) $Y_{5C} = 72.6/[1 + \exp[-(d - 10.0)/2.7]]$, $R^2 = 0.92$ and $Y_{30C} = 84.7/[1 + \exp[-(d - 8.5)/2.6]]$, $R^2 = 0.94$; (G) $Y_{5C} = 55.7/[1 + \exp[-(d - 11.2)/3.1]]$, $R^2 = 0.96$ and $Y_{30C} = 73.0/[1 + \exp[-(d - 9.2)/3.0]]$, $R^2 = 0.94$; (H) $Y_{5C} = 61.6/[1 + \exp[-(d - 12.0)/3.7]]$, $R^2 = 0.90$ and $Y_{30C} = 70.5/[1 + \exp[-(d - 10.8)/3.5]]$, $R^2 = 0.85$; (I) $Y_{5C} = 65.4/[1 + \exp[-(d - 14.7)/4.8]]$, $R^2 = 0.84$ and $Y_{30C} = 11.9/[1 + \exp[-(d - 16.5)/4.7]]$, $R^2 = 0.33$; (J) $Y_{5C} = 58.1/[1 + \exp[-(d - 12.3)/4.1]]$, $R^2 = 0.87$; (K) $Y_{5C} = 76.8/[1 + \exp[-(d - 9.5)/2.3]]$, $R^2 = 0.95$ and $Y_{30C} = 87.4/[1 + \exp[-(d - 9.0)/2.3]]$, $R^2 = 0.97$; (L) $Y_{5C} = 61.7/[1 + \exp[-(d - 11.8)/3.2]]$, $R^2 = 0.89$ and $Y_{30C} = 68.3/[1 + \exp[-(d - 9.9)/2.7]]$, $R^2 = 0.94$; (M) $Y_{5C} = 55.8/[1 + \exp[-(d - 10.3)/2.7]]$, $R^2 = 0.92$ and $Y_{30C} = 69.3/[1 + \exp[-(d - 9.3)/2.5]]$, $R^2 = 0.93$; (N) $Y_{5C} = 50.1/[1 + \exp[-(d - 10.8)/2.7]]$, $R^2 = 0.82$; (O) $Y_{5C} = 54.7/[1 + \exp[-(d - 11.1)/3.0]]$, $R^2 = 0.93$.

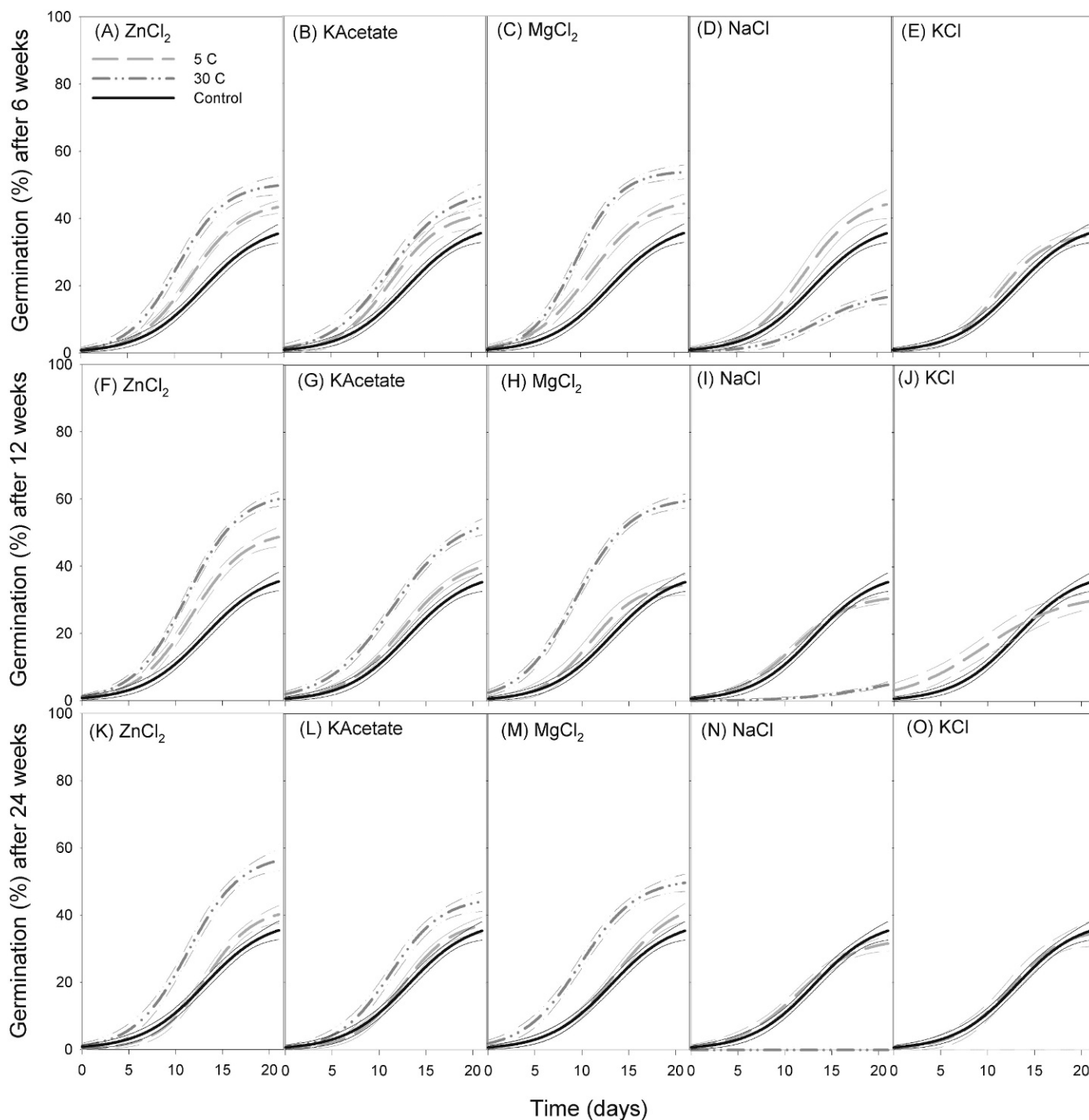


Figure 3. Predicted germination of leafy spurge seeds from the Fargo population afterripened for (A–E) 6 wk, (F–J) 12 wk, and (K–O) 24 wk at 5 and 30 C with different seed moisture levels provided by saturated salt solutions (see Table 1). The nonafterripened control is included for comparison. The 95% confidence intervals are depicted by the narrow lines. Equations are (A) $Y_{5C} = 44.9/[1 + \exp\{-(d - 11.7)/2.8\}]$, $R^2 = 0.94$ and $Y_{30C} = 50.6/[1 + \exp\{-(d - 10.2)/2.6\}]$, $R^2 = 0.89$; (B) $Y_{5C} = 42.0/[1 + \exp\{-(d - 11.4)/2.8\}]$, $R^2 = 0.74$ and $Y_{30C} = 48.3/[1 + \exp\{-(d - 11.2)/3.2\}]$, $R^2 = 0.80$; (C) $Y_{5C} = 45.9/[1 + \exp\{-(d - 10.9)/3.1\}]$, $R^2 = 0.87$ and $Y_{30C} = 54.0/[1 + \exp\{-(d - 9.4)/2.4\}]$, $R^2 = 0.93$; (D) $Y_{5C} = 46.3/[1 + \exp\{-(d - 12.1)/3.0\}]$, $R^2 = 0.77$ and $Y_{30C} = 17.9/[1 + \exp\{-(d - 13.6)/3.0\}]$, $R^2 = 0.67$; (E) $Y_{5C} = 35.6/[1 + \exp\{-(d - 11.3)/2.6\}]$, $R^2 = 0.85$; (F) $Y_{5C} = 50.7/[1 + \exp\{-(d - 11.7)/2.9\}]$, $R^2 = 0.90$ and $Y_{30C} = 62.0/[1 + \exp\{-(d - 11.2)/2.9\}]$, $R^2 = 0.95$; (G) $Y_{5C} = 42.8/[1 + \exp\{-(d - 12.5)/3.2\}]$, $R^2 = 0.92$ and $Y_{30C} = 55.2/[1 + \exp\{-(d - 11.4)/3.5\}]$, $R^2 = 0.93$; (H) $Y_{5C} = 35.4/[1 + \exp\{-(d - 11.0)/2.7\}]$, $R^2 = 0.77$ and $Y_{30C} = 60.7/[1 + \exp\{-(d - 9.3)/3.0\}]$, $R^2 = 0.94$; (I) $Y_{5C} = 31.6/[1 + \exp\{-(d - 10.8)/3.1\}]$, $R^2 = 0.94$ and $Y_{30C} = 10.7/[1 + \exp\{-(d - 21.8)/4.7\}]$, $R^2 = 0.53$; (J) $Y_{5C} = 35.9/[1 + \exp\{-(d - 10.3)/3.3\}]$, $R^2 = 0.84$; (K) $Y_{5C} = 42.1/[1 + \exp\{-(d - 13.2)/2.6\}]$, $R^2 = 0.89$ and $Y_{30C} = 58.2/[1 + \exp\{-(d - 11.3)/2.9\}]$, $R^2 = 0.90$; (L) $Y_{5C} = 38.6/[1 + \exp\{-(d - 12.5)/2.7\}]$, $R^2 = 0.88$ and $Y_{30C} = 45.4/[1 + \exp\{-(d - 11.2)/2.8\}]$, $R^2 = 0.86$; (M) $Y_{5C} = 45.1/[1 + \exp\{-(d - 13.6)/3.3\}]$, $R^2 = 0.88$ and $Y_{30C} = 51.0/[1 + \exp\{-(d - 9.8)/3.0\}]$, $R^2 = 0.90$; (N) $Y_{5C} = 33.2/[1 + \exp\{-(d - 11.3)/3.2\}]$, $R^2 = 0.84$; (O) $Y_{5C} = 36.2/[1 + \exp\{-(d - 12.3)/2.9\}]$, $R^2 = 0.73$.

Brown and Porter 1942; Krueger and Shaner 1982). The higher temperature was selected because wild poinsettia seeds responded to afterripening at 36 C under low seed moisture conditions (Bannon et al. 1978). There were differences in germination between populations and all treatments provided a greater rate of germination than the control, as seen in the initial experiment. However, there was little or no difference among afterripening temperatures (data not shown), except germination was reduced slightly by afterripening at 5 C for Fargo population seeds.

Afterripening of leafy spurge seeds harvested from the Fargo population in 1999 and 2000 was done previously at 5 and 20 C using seeds with relatively "high" and "low" moisture. High seed moisture levels were attained by stratification in moist sand and low seed moisture by incubating dry seeds under prevailing ambient conditions. Seeds afterripened for 24 wk at 20 C under high moisture conditions displayed approximately 94% germination in 21 d, whereas afterripening at 20 C under low moisture resulted in 70% germination (Foley 2004). Seeds harvested in 2000 and afterripened at 5 C under moist and dry conditions displayed 91 and 70% germination, respectively, but germination was 40 to 50 percentage points lower for the seeds harvested in 1999.

Small changes in temperature and seed moisture during afterripening can have large effects on subsequent germination (Esashi et al. 1993; Leopold et al. 1988). Therefore, direct comparison of results from the past and current experiments is not possible because of difference in the high afterripening temperature and the undefined levels of seed moisture provide by stratification treatments (Foley 2004). Nevertheless, some generalizations are possible. For example, seeds from both experiments afterripened uniformly when exposed to relatively warm temperatures of 20 to 30 C and dry to low seed moisture conditions. Warm, moist conditions for 12 to 24 wk provided for a high level of afterripening in the first experiment (Foley 2004), whereas in the current experiment warm, moist conditions (30 C and \geq 9% seed moisture) resulted in loss of seed viability (Figures 2 and 3). The difference in germination between the two experiments following afterripening under warm, moist conditions might be explained by an inverse relationship between temperature and seed moisture content for afterripening as seen for wild oat (Foley 1994). In such a case, the average seed moisture content at 20 C over 24 wk would have been sufficiently low to be conducive to afterripening rather than ageing. Indeed, the lower afterripening temperature of 5 C allows for relatively high seed moisture content (13%) without ageing and a seed moisture content of 5.6% at 30 C facilitated afterripening but not ageing (Figure 2C, 2H, and 2M and 3C, 3H, and 3M).

In contrast with the previous experiment where afterripening dry and high moisture seeds at 5 C stimulated germination, albeit to varying levels depending on the year harvested (Foley 2004), only a seed moisture content of 3.3% stimulated germination of Harwood and Fargo population seeds afterripened at 5 C (Figure 2A, 2F and 2K and 3A, 3F, and 3K). The inconsistency in effects between experiments, particularly the robust germination following cool, moist afterripening of Fargo population seeds harvested in 2000, is difficult to reconcile with current findings because average seed moisture during stratification would be expected to be much greater than 3.3%. It is likely that seed moisture levels

greater than 13% are required for afterripening at 5 C because both leafy spurge and prostrate spurge responded to stratification at 5 C (Foley 2004; Krueger and Shaner 1982).

Long-term management of leafy spurge requires knowledge on the biology of leafy spurge seeds because they are dominant in the soil of infested areas (Kirby et al. 2003) and have some degree of longevity. Bowes and Thomas (1978) estimated annual loss of leafy spurge seeds from the soil seed bank at 13% of the original population per year. It is evident from this research that a small change in temperature or seed moisture during afterripening provides for complex regulation of germination and viability of seeds within populations of leafy spurge. Questions concerning the interaction of a greater range of temperatures and seed moisture conditions that promote optimum afterripening or loss of viability and the physiological basis for these interactions remain to be answered.

Sources of Materials

¹ South Dakota seed blower, Seedbuco Equipment Co., 1022 W. Jackson Blvd., Chicago, IL 60607.

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